

ABSTRACT OF THE DISCLOSURE

A method and kit for synthesizing a nucleic acid molecule comprising at least one non-canonical nucleoside triphosphate using a double-mutant polymerase having a reduced discrimination between canonical and non-canonical substrates is disclosed. The method comprises incubating a template nucleic acid in a reaction mixture comprising the mutant nucleic acid polymerase and the appropriate canonical and non-canonical nucleoside triphosphates which are desired substrates for the mutant nucleic acid polymerase. The present invention is also a method of determining the sequence of a nucleic acid molecule using the mutant polymerase to create a nucleic acid molecule comprising at least one non-canonical nucleoside triphosphate.

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